Diazotrophic activity and denitrification in two long-term chronosequences of maritime Antarctica

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HIGHLIGHTS

- Both chronosequences showed contrasting patterns of diazotrophic activity and denitrification.
- Higher diazotrophic activity was linked to higher abundance of *Cyanobacteria*.
- Higher denitrification was linked to higher abundance of *Rhodanobacter*.
- Passive warming and water addition increased diazotrophic activity.
- Denitrification was no responsive to either passive warming or water addition.

GRAPHICAL ABSTRACT
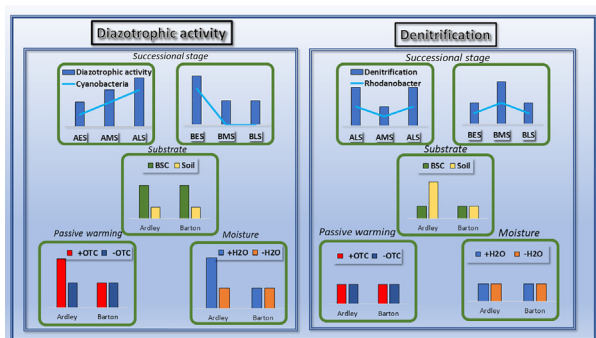
ABSTRACT

The main goals of this study were to identify whether key processes involved in microbial soil nitrogen transformations, such as diazotrophic activity and denitrification, the chemical properties of limiting elements in the soil, and microbial community structure, differ in the different successional stages of two long term chronosequences in maritime Antarctica. Moreover, we expect the rates of diazotrophic activity and denitrification to be stimulated by increases in air temperature and moisture. To answer these questions, we selected three stages in the succession (early, mid and late) in each of two well established chronosequences: three raised beaches in Ardley Island; and the Barton Peninsula, which includes two cosmogenically dated sites and the forefield of the Fourcade glacier. In the Ardley chronosequence, higher diazotrophic activity was found in the older successional stages, concomitant with an increase in the abundance of *Cyanobacteria*. In the Barton chronosequence, *Cyanobacteria* were only present and abundant (*Microcoleus*) in the early successional stage, coinciding with the highest diazotrophic activity. Denitrification in the Barton chronosequence tended to be highest at the mid successional sites, associated with the highest abundance of *Rhodanobacter*. In the Ardley chronosequence, the lowest abundance of *Rhodanobacter* was linked to lower denitrification rates in the mid successional stage. In the Ardley chronosequence, significant positive effects of passive warming and water addition on diazotrophic activity were detected in the first and the second years of the study respectively. In the Barton chronosequence on the other hand, there was no response to either passive warming or water addition, probably a manifestation of the higher nutrient limitation in this site. Denitrification showed no response to either warming or water addition. Thus, the response of microbial nitrogen transformations to global change is highly dependent on the environmental setting, such as soil origin, age and climate regime.

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1. Introduction

The Antarctic Peninsula is continually undergoing profound geomorphological changes due to the intrinsic climatic dynamics that have occurred since the Last Glacial Maximum (Cofaigh et al., 2014; Fretwell et al., 2010; Watcham et al., 2011). Historical satellite images indicate that active glacier-thinning and retreat has been occurring over the last five decades (Rückamp et al., 2011). In southern King George Island, the substrates of the deglaciated landscape of glacier forefields and raised beaches (palaeo-beaches) are well dated (Cofaigh et al., 2014). This allows us to reconstruct chronosequences through which to study ecosystem development, using the principle of space-for-time substitution (Pickett, 1989).

Since the 1950s, there has been a growing interest in investigating glacier forefields around the world. These studies document deterministic patterns of change in ecosystem properties and functions, as well as microbial community structures with pedogenesis (Bradley et al., 2014 and references therein). Increases in vegetation cover and soil microbial biomass, contribute to the build-up of organic matter in the sediments of the glacier forefield (Göransson et al., 2011, Schurig et al., 2013, Mori et al., 2017) and the concomitant decreases in soil pH, as organic acids increase with soil age (Mateos-Rivera et al., 2016; Kim et al., 2017; Alfaro et al., 2020). Moreover, bacterial communities, which are strongly dependent on soil carbon (C) and nitrogen (N), such as heterotrophic and autotrophic denitrifiers and diazotrophs, respond to changes in soil content of these elements, presenting alterations in their community composition and structure with soil age (Rime et al., 2015; Castle et al., 2016; Fernandez-Martinez et al., 2017; Kim et al., 2017; Pessi et al., 2015; Jiang et al., 2018). The direction of change of microbial functions and soil chemical properties is not universal for global ecosystems but depends on state factors – mainly the geological substrate (e.g., volcanic vs. sedimentary rocks) and environmental setting (e.g., dry vs. wet environments) (Delgado-Baquerizo et al., 2019; Delgado- Baquerizo et al., 2020). Although little is known about ecosystem development in the very limited ice-free regions of Antarctica (0.44%), these studies confirm the diverse directional pattern of changes in soil properties and microbial community composition and structure (Strauss et al., 2009, Bajerski and Wagner, 2013, Pessi et al., 2015, Boy et al., 2016, Yan et al., 2017, Garrido-Benavent et al., 2020, Krauze et al., 2021). However, less is known about the changes in microbial activity involved in soil N transformations, such as diazotrophic activity and denitrification, and even less about how these functions will be affected, either positively or negatively, by the predicted increases in air temperature (0.5–1.5 °C) and mean annual precipitation (5–10%) for maritime Antarctica in the next 4 decades (Bozkurt et al., 2021).

In the present study, we focus on two soil microbial N transformations involved in the nitrogen cycle: nitrogen fixation performed by diazotrophic bacteria; and denitrification performed by denitrifiers endowed with the nitrogenase and nitrate reductase enzymes, respectively. N fixation is a key ecosystem function in pristine and newly exposed substrates, through which new N is acquired by ecosystems. In contrast, denitrification is an important cause of N loss within ecosystems and a source of nitrous oxide, which is a potent greenhouse gas.

Our main hypothesis is that diazotrophic activity and denitrification, the chemical properties of limiting elements in the soil, and microbial community structure, will differ in the different successional stages. Moreover, we expect that the rates of diazotrophic activity and denitrification will be stimulated by increases in air temperature and soil humidity. The answers to these questions will help to explain how Antarctic ecosystems will respond to climate change. In order to test our hypotheses, we selected three stages (early, mid and late) in the successions in each of two well established chronosequences: three raised beaches in Ardley Island (Fretwell et al., 2010); and the Barton Peninsula, which includes two cosmogenically dated sites (Seong et al., 2009) and the forefield of the Fourcade glacier. Our research goals were: i) to describe the trends in chemical properties of soils, such as pH, available N and phosphorous (P), base cations, and total contents of N, P and C; ii) to measure the rates of microbial nitrogen transformations, including diazotrophic activity and denitrification, occurring in soils and biological soil crusts (BSCs); iii) to estimate soil microbial diversity and abundance, and their relationships with the rates of different microbial N transformations and chemical properties of soils; and iv) to determine the effect of passive experimental warming and moisture on microbial nitrogen transformations.

The novelty of this study lies in providing knowledge about the patterns of change with the development of ecosystems in diazotrophic activity and denitrification, the microbial assembly of the soils, and the soil bacteria that participate in these ecosystem functions. Moreover, experimental warming and moisture increases will give us insights into how key microbial N transformations, such as N-fixation and denitrification, will respond to the climate change now being experienced in the Antarctic Peninsula.

2. Methodology

2.1. Study sites

In order to study how microbial N transformations and microbial assemblages develop with soil age, we selected two well established chronosequences in southern King George Island, maritime Antarctica: 1) a chronosequence of three raised beaches in Ardley Island (62°13′S, 58°56′ W), where early (AES: 200–650 years BP), mid (AMS: 2500–4400 years BP) and late (ALS: 7200 years BP) successional stages were selected, dated according to an isobase model (Fretwell et al., 2010); and 2) a chronosequence in Barton Peninsula (62°23′S, 58°78′W), which consisted of an early successional stage located about 20 m from the glacier terminus of the Fourcade glacier (BES: 8–10 years BP), and two sites dated by cosmogenic [36]chloride (Seong et al., 2009), viz. a mid successional stage (BMS: 5000 ± 1200 years BP), and a late stage (BLS: 11900 ± 2300 years BP) (Table 1). The vegetation associations found in Ardley Island and the Barton Peninsula are similar, mainly composed of the lichen/moss genera *Usnea-Andreaea-Himantormia*; except for the early succession stage in the Fourcade glacier, which is dominated by sparse cover of the moss *Bryum* sp. (Peter et al., 2008; Gyeong et al., 2021) (Table 1). Records of the "Eduardo Frei Montalva" weather station, located about 2 km from Ardley Island, indicate mean annual temperature of − 2.4 °C and mean annual precipitation of 737.7 mm in the last decade. In Barton Peninsula, the five-decade record indicates mean annual temperature of − 1.8 °C and mean annual precipitation of 437.6 mm. However, Lee et al. (2008) document a

Table 1
Study sites in Ardley Island and Barton Peninsula, King George Island, maritime Antarctica.

Successional stage	Latitude S	Longitude	Exposure time Years BP	Vegetation	Geomorphology	Altitude (m.a.s.l.)
Ardley Island						
Early (AES)	62.213	58.947	200–650	*Usnea-Himantormia*	Palaeo-beach	5
Mid (AMS)	62.213	58.946	2500–4400	*Usnea-Himantormia*	Palaeo-beach	10
Late (ALS)	62.212	58.945	7200	*Usnea-Himantormia*	Palaeo-beach	20
Barton Peninsula						
Early (BES)	62.229	58.712	8–10	*Bryum* sp.	Glacial till	10
Mid (BMS)	62.225	58.779	5000 ± 1200	*Usnea-Himantormia-Adreae*	Patterned ground	105
Late (BLS)	62.227	58.771	11,900 ± 2300	*Usnea-Himantormia*	Patterned ground	165

progressive increase in both temperature and precipitation over the last two decades. The soil type of the palaeo-beaches of Ardley Island can be classified as Turbic-Leptic-Cryosol (Michel et al., 2014); in the Barton Peninsula, the mid and late successional stages correspond to Glacic and Haplic Turbic Cryosol respectively (Lopes et al., 2019), while the soil in the Fourcade glacier site is Glacial Till.

2.2. Microbial nitrogen transformations

2.2.1. Diazotrophic activity

In order to estimate diazotrophic activity, the enzymatic activity of nitrogenase was measured in two types of substrates: i) biological soil crusts (BSCs) composed of lichens and bryophytes, where diazotrophs are present as symbiotic organisms or as epiphylls; and ii) free-living diazotrophs present in soils. In both chronosequences and in each successional stage, seven random samples were taken of: a) BSCs (3–4 g dry weight); and b) soil (6 cm^3 pvc soil cores). Diazotrophic activity was determined using the acetylene reduction assay (Myrold et al., 1999). Samples were incubated in situ, for a maximum of 3 days, using hermetically closed 130 ml glass jars containing a mixture of air and acetylene at 10% v/v. An additional sample per treatment from each substrate type was incubated without acetylene as a control. One gas sample per jar was taken every day and stored in 4 ml Becton-Dickinson Vacutainers™; these samples were subsequently analysed for ethylene production in a Shimadzu gas chromatograph GC-8AIF (Kyoto, Japan) equipped with a steel column filled with Porapak N (SupelcoTM, 1 m*1/400ss, 80/100 mesh (Bellefonte, Pennsylvania, USA), and with a FID detector. The ethylene concentration was determined from a calibration curve by diluting 100 ppm ethylene balance in nitrogen using Scott™ analysis gases (Bellefonte, Pennsylvania, USA). Acetylene reduction activity, an estimation of diazotrophic activity, was obtained from the slope of the linear fit of ethylene production during incubation in 130 ml headspace and referred to dry weight.

2.2.2. Denitrification

In order to estimate denitrification rates, the enzymatic activity of nitrate reductase was measured in the same sample types described above, incubated in the laboratory at Escudero Base, the Chilean Scientific Research Station on Fildes Peninsula, King George Island. Denitrification rates were determined with the acetylene inhibition assay in intact soil cores and BSCs (Groffman et al., 1999). This method was selected because nitrous oxide (N_2O) reductase is inhibited by acetylene, allowing the accumulation of nitrous oxide in an acetylene atmosphere, which can be measured by gas chromatography. Soil samples were placed inside 130 ml hermetic glass jars and incubated for 6 h under a 10% v/v acetylene atmosphere. Gas samples were taken at 2 and 6 h and stored in a 4 ml vacutainer. The N_2O concentrations in the gas samples were determined in a Shimadzu gas chromatograph equipped with a Porapack column Q 80/100 and electron capture detector. Calibration curves were prepared with a 1 ppm nitrous oxide balance of nitrogen using Scott analysis gases. Denitrification rates were estimated from the N_2O-N concentration difference between time 2 and time 6, and expressed as an area.

2.3. Passive warming and water addition experiments

To passively increase the temperature conditions, five open-top chambers (OTCs) were installed in each of the six successional stages in February 2019. These OTCs are hexagonal in shape, constructed of perforated transparent acrylic panels to avoid excessive heating (Sáez et al., 2018). The chambers are 40 cm in height with base diameter of 115 cm, and 25 perforations of 1.5 cm diameter each (Fig. 1S). To evaluate the effect of passive warming and moisture availability on the rates of microbial nitrogen transformations, the glass jars from experiments 2a and 2b were incubated in situ and in the laboratory, respectively, during the southern summer, i.e. February 2019 and February 2021, using the following treatments: a) open-air, outside and next to the OTCs; b) inside standard OTCs; c) with no addition of deionized water (hereafter DIW); d) with addition
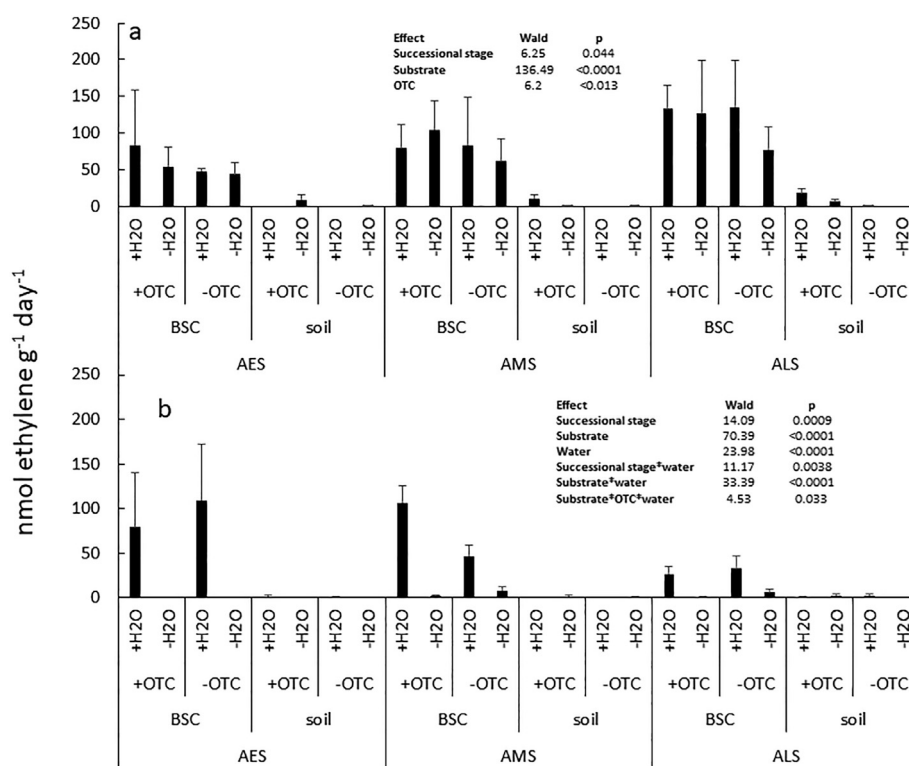

Fig. 1. Mean ($n = 6 \pm SD$) diazotrophic activity (the rate of acetylene reduced to ethylene) in the three successional stages of the Ardley chronosequence in the BSC and soil inside (+OTC) and outside (−OTC) the open-top chambers and with (+H$_2$O) and without (−H$_2$O) water addition, during (a) February 2019 and (b) February 2021. Only the significant effects of Wald statistics are shown in the box (Tables 2S and 3S).

of 3 ml DIW. The water addition experiment proceeded by adding 3 ml of DIW to samples, thereby increasing the water content on average to about 1.5 times the field water content.

The experiment design corresponded to randomized block design (Quinn and Keough, 2002). For each OTC, consisting of a block or set of treatments, two samples of each type of substrate (CBS, soil) were placed inside the jars. One was treated with 3 ml of deionized water (DIW) and the other was left at field moisture content. Therefore, there were a total of four jars inside each OTC. This procedure was replicated outside the OTCs, making a total of eight samples per block. Additionally, as backup samples, in one of the five OTCs, an additional set of eight treatments was included, and an extra OTC was used as a control (without the addition of acetylene). Therefore, there were a total of seven blocks (6 blocks with acetylene and one control block), with eight treatments in each, making a total of 56 samples per successional stage.

In each successional stage, two replicates of data loggers were installed on the soil surface to record temperature and relative humidity inside and outside the chambers. The experiments at both study sites, Barton Peninsula and Ardley Island, were conducted in February (southern summer) 2019 and 2021 and the chambers were left until 2021. The only exception was the late successional stage on the Barton Peninsula, where the OTCs were blown down by the wind. In 2019, all experiments measuring diazotrophic activity were conducted in the field. In 2021, experiments on the samples of the mid and early successional stages of the Barton Peninsula were conducted at the laboratory of the Escudero Base.

2.4. Soil sampling and chemical analysis

Five random samples of surface soils (0–5 cm) were taken from each successional stage in both chronosequences. Soils were sieved using a 2 mm mesh size prior to chemical analysis. Plant-available nitrogen was extracted in a 0.021 mol/L KAl(SO$_4$)$_2$ solution (1:4) and the amounts of available N were determined as ammonium and nitrate by means of fractionated steam distillation (Pérez et al., 1998). Plant-available P was extracted by lactation using the CAL (Calcium-Acetate-Lactate) method and determined colorimetrically using the Molybdenum Blue method. The water contents of the soil samples were determined gravimetrically. Soil reaction was determined with a pH electrode in a 1:2 soil:water suspension. Exchangeable base cations of calcium, sodium, potassium and magnesium were recovered from fresh soils in a 1 M ammonium acetate solution (1:10), and determined using a Perkin Elmer 2380 AAS (Robertson et al., 1999). The dry samples of soil were ground for determination of total N and C by means of flash combustion using an NA2500 Carlo Erba Element Analyzer (Sollins et al., 1999). Phosphorus from ground soil was extracted with concentrated sulphuric acid together with a water peroxide solution in a Hach Digesdahl digester and determined by the Molybdenum Blue method (Kuo, 1996).

2.5. Microbial assemblages in soils

In February 2019, up to five surface soil samples (0-5 cm) were taken at random from each successional stage in both chronosequences using a hand trowel, which was sterilized by 70% alcohol prior to sampling. Soils were passed through a 2-mm sieve and stored at −20 °C during shipment to the KOPRI laboratory in South Korea. Total DNA was extracted from 0.3 g of each soil sample using the DNeasy PowerSoil kit (QIAGEN) according to manufacturer's protocol, and the extracted DNA was further purified by OneStep PCR Inhibitor Removal kit (Zymo Research) to improve DNA quality. DNA was amplified with universal primers (515FB/926R) targeting the V4-V5 region of 16S rRNA gene (Parada et al., 2016). 16S PCR using dual-indexed fusion primers, library preparation, and amplicon sequencing (MiSeq 2 × 300 bp) were carried out at Integrated Microbiome Resource (IMR). All the details of wet-lab protocols are found at Microbiome Helper GitHub (https://github.com/LangilleLab/microbiome_helper/wiki/Microbiome-Amplicon-Sequencing-Workflow). Raw sequencing data were processed using the DADA2 algorithm (Callahan et al., 2016) to

infer amplicon sequence variants (ASVs), and the same parameter setting was used as in the previous paper (Gyeong et al., 2021). The ASV representative sequences were taxonomically classified by matching them against the EzBioCloud 16S database (Yoon et al., 2017) using the Naïve Bayesian Classifier with a confidence threshold of 80%. The α-diversity was determined by the Shannon index with the normalized dataset by rarefying sequences to the smallest sample size (6967 sequences per sample). The β-diversity was determined by calculating Bray−Curtis dissimilarities of all pairs of samples using the Hellinger-transformed ASV abundance matrix, and visualized using non-metric multidimensional scaling (NMDS). Raw sequence data were submitted to the NCBI Sequence Read Archive (SRA) database with the accession number: **PRJNA759713** (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA759713).

2.6. Statistical analysis

The statistically significant differences in chemical properties of soils between chronosequences and successional stages were evaluated by two-way ANOVA and *a-posteriori* Tukey tests, before testing for the homogeneity of variances. Where the null hypothesis was rejected, the data were log transformed. One-way ANOVA and a posteriori Tukey test were applied to test for the significant differences between successional stages in the Shannon index. As the data were not normally distributed, statistically significant differences in temperatures inside vs. outside the OTCs were evaluated with non-parametric Mann–Whitney *U* tests. For the same reason, the effects of the successional stage (early, mid, late), type of substrate (BSC vs soil), passive warming (+ OTC vs − OTC) and water addition (+H$_2$O vs −H$_2$O) on diazotrophic activity (acetylene reduction rates) and denitrification rates were evaluated by Nonparametric Methods in General Factorial Designs with the package "rankFD" in R version 4.1.1. (Brunner et al., 2017).

3. Results

3.1. Chemical properties of soils

The chemical properties of the soils differed significantly between the two chronosequences. The Ardley chronosequence presented higher water content ($F_{1,27}$ = 306.1; $p < 0.001$) and contents of base cations ($F_{1,26}$ = 95.85; $p < 0.001$), available P ($F_{1,27}$ = 17.87; $p < 0.001$), and total N ($F_{1,27}$ = 45.02; $p < 0.001$) and C ($F_{1,27}$ = 144.79; $p < 0.001$) than the soils of the Barton chronosequence. On the other hand, the soils of the Barton chronosequence presented higher pH values ($F_{1,26}$ = 214.44; $p < 0.001$) and total P ($F_{1,27}$ = 35.22; $p < 0.001$) than the soils of the Ardley chronosequence. Interactive effects of site*successional stage and Tukey tests indicated that in the Ardley chronosequence, AES presented significantly higher contents of base cations than ALS, which in turn presented a higher total P content than AES and AMS (Table 2). In the Barton chronosequence, significantly higher contents of C and water were observed in BMS, lower in BLS and lowest in BES (Table 2). The pH value observed was highest in BES, significantly lower in BLS and lowest in BMS. A higher content of base cations was observed in BES than in either BMS or BLS. Available P was the highest in BMS followed by BES, and lowest in BLS. The contents of total and available N were higher in BMS than in BES and BLS. Total P was similar in the three successional stages.

3.2. Effects of the OTC on microclimatic conditions

The OTC significantly increased the mean surface air temperature by 0.5–0.9 °C and the minimum by 0.64–2.18 °C. Increases in the maximum temperature by 0.04–1.3 °C were also obtained; however, these differences were not statistically significant (Table 3, Table 1S). The relative humidity inside and outside the OTCs was over 96% in all sites, except for BES where the relative humidity at the surface was over 85% both inside and outside the OTC.

Table 2

Chemical characteristics of soils in the three successional stages in the Ardley and Barton chronosequences. In parenthesis are the SD from the mean values, $n = 5$. Different capital letters indicate significant differences among chronosequences and lower-case letter among successional stages within a chronosequence.

	Water content (%)	pH (H$_2$O)	Available N mg/kg	Available P mg/kg	Base cations cmol/kg	%N	% C	%P
Ardley	A	B		A	A	A	A	
AES	54.47	5.54	29.57	22.96	35.76a	2.23	27.61	0.04b
	(2.9)	(0.05)	(3.87)	(2.22)	(11.09)	(0.12)	(2.1)	(0.0)
AMS	50.86	5.3	25.03	24.25	26.25ab	2.27	28.29	0.04b
	(2.4)	(0.05)	(3.67)	(1.08)	(10.32)	(0.2)	(28.29)	(0.01)
ALS	45.99	5.45	25.00	26.6	17.99b	1.8	20.97	0.14a
	(2.16)	(0.06)	(3.39)	(0.65)	(4.49)	(0.1)	(1.16)	(0.02)
Barton	B	A		B	B	B	B	
BES	8.28c	8.88a	15.27b	7.56b	16.69a	0.01b	0.10c	0.17
	(1.32)	(0.25)	(4.15)	(1.45)	(1.77)	(0.02)	(0.21)	(0.03)
BMS	52.23a	4.94c	58.4a	129.87a	5.6b	1.65a	15.61a	0.3
	(12.56)	(0.23)	(19.58)	(17.66)	(2.52)	(0.94)	(8.21)	(0.12)
BLS	21.65b	6.1b	22.95b	2.73c	5.19b	0.21b	2.76b	0.13
	(1.49)	(0.27)	(16.34)	(2.5)	(1.33)	(0.09)	(1.36)	(0.09)

3.3. Diazotrophic activity

In the Ardley chronosequence, in 2019 there was a significant effect of successional stage, substrate and passive warming on diazotrophic activity. Lower rates were found in AES than ALS (Fig. 1a, Table 2S). Higher rates were found under passive warming and the BSCs presented higher rates than the soils. No interactive effects were detected (Table 2S). In 2021, the significant effects of successional stage and substrate were maintained (Table 3S). While no effect of passive warming was observed, there was a significant effect of water addition on diazotrophic activity (Fig. 1b, Table 3S). Lower diazotrophic activity was found in AES than AMS. Significant interactive effects of successional stage*substrate and substrate*water indicate that water addition significantly increased diazotrophic activity in the BSCs of AES and AMS (Fig. 1b, Table 3S).

In the Barton chronosequence, in 2019 there was a significant effect of successional stage and substrate on diazotrophic activity (Fig. 2a, Table 4S). No effect of either passive warming or water addition was observed. In 2021, the significant effects of successional stage and substrate were maintained, being highest in the BSCs of BES (Fig. 2b Table 5S). In addition, significant interactive effects of successional stage ∗ passive warming ∗ water were observed, indicating that in BMS a positive effect of passive warming without water addition; however passive warming had a negative effect when combined with water addition (Table 5S).

3.4. Denitrification

In Ardley Island in 2019 there was a significant effect of successional stage on denitrification rates: AMS presented the lowest denitrification rate, followed by AES, and the highest was found in ALS (Fig. 3a, Table 6S). No effect was found of substrate, passive warming or water addition on denitrification rates (Table 6S). In 2021 there was a significant effect of successional stage and substrate on denitrification rates: AMS presented a lower rate than ALS and soil presented a higher rate than BSCs. Interactive effects of successional stage ∗ substrate indicate that the

Table 3

Mean surface air temperature (°C) and relative humidity (%) reached inside and outside the OTCs in Ardley Island and Barton Peninsula from February 2019 until February 2021. The difference between the two treatments is indicated in italics. Different letters indicate significant differences ($P < 0.05$) in inside and outside temperatures (Table 1S).

|	Mean temperature	Mean maximum temperature	Mean minimum temperature	Mean relative humidity	Mean maximum relative humidity	Mean minimum relative humidity
AES						
Inside	−0.06a	2.35a	−1.44a	96.64	97.80	94.33
Outside	−0.95b	1.84b	−2.98b	96.87	99.45	91.77
Difference	0.89	0.50	1.53	−0.23	−1.65	2.56
AMS						
Inside	0.15a	2.65	−1.28a	98.64	99.57	96.57
Outside	−0.35b	1.73	−1.92b	97.63	99.62	93.84
Difference	0.50	0.92	0.64	1.01	−0.05	2.73
ALS						
Inside	−0.23a	2.32	−1.47a	99.30	99.82	97.94
Outside	−1.12b	1.80	−3.64b	96.64	99.54	90.22
Difference	0.90	0.52	2.18	2.66	0.29	7.72
BES						
Inside	1.17a	3.78	−0.41a	85.49	86.63	83.73
Outside	0.44b	2.48	−1.03b	89.86	92.36	86.31
Difference	0.73	1.30	0.62	−4.37	−5.73	−2.58
BMS						
Inside	−0.66a	1.51	−1.86a	97.79	99.20	94.78
Outside	−1.15b	1.47	−3.15b	95.47	97.48	91.90
Difference	0.49	0.04	1.28	2.32	1.73	2.88
BLS						
Outside[a]	−2.06	0.63	−4.03	97.09	99.45	92.49

[a] Only outside records as the OTCs were blown down.
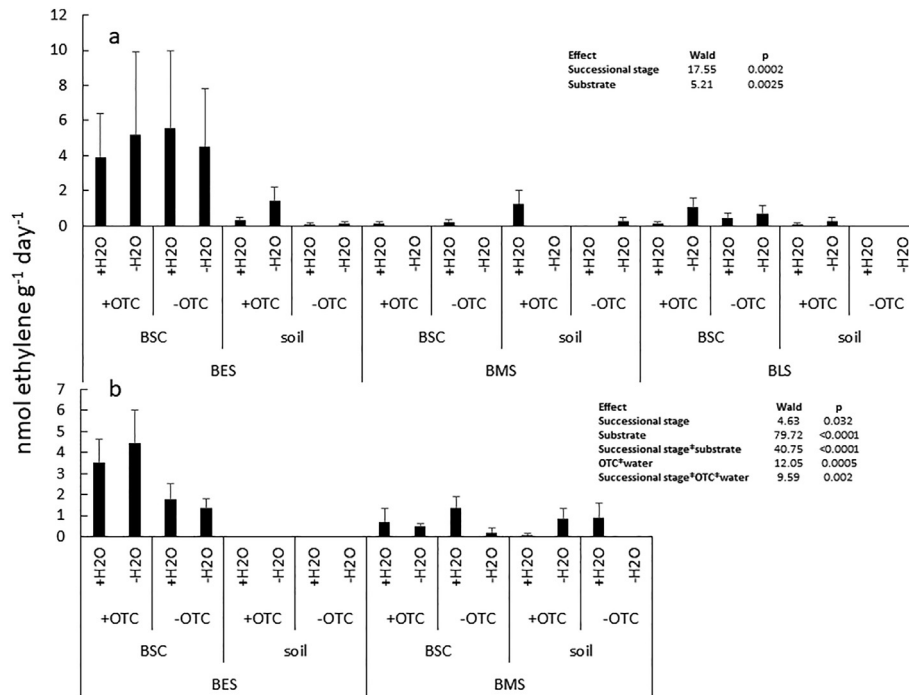
Fig. 2. Mean (n = 6 ± SD) diazotrophic activity (the rate of acetylene reduced to ethylene) in field experiments in the three successional stages of the Barton chronosequence in the BSC and soil inside (+OTC) and outside (−OTC) the open-top chambers and with (+H_2O) and without (−H_2O) water addition, during (a) February 2019 and (b) February 2021. In 2021, only BES and BMS were measured. Only the significant effects of Wald statistics are shown in the box (Tables 4S and 5S).
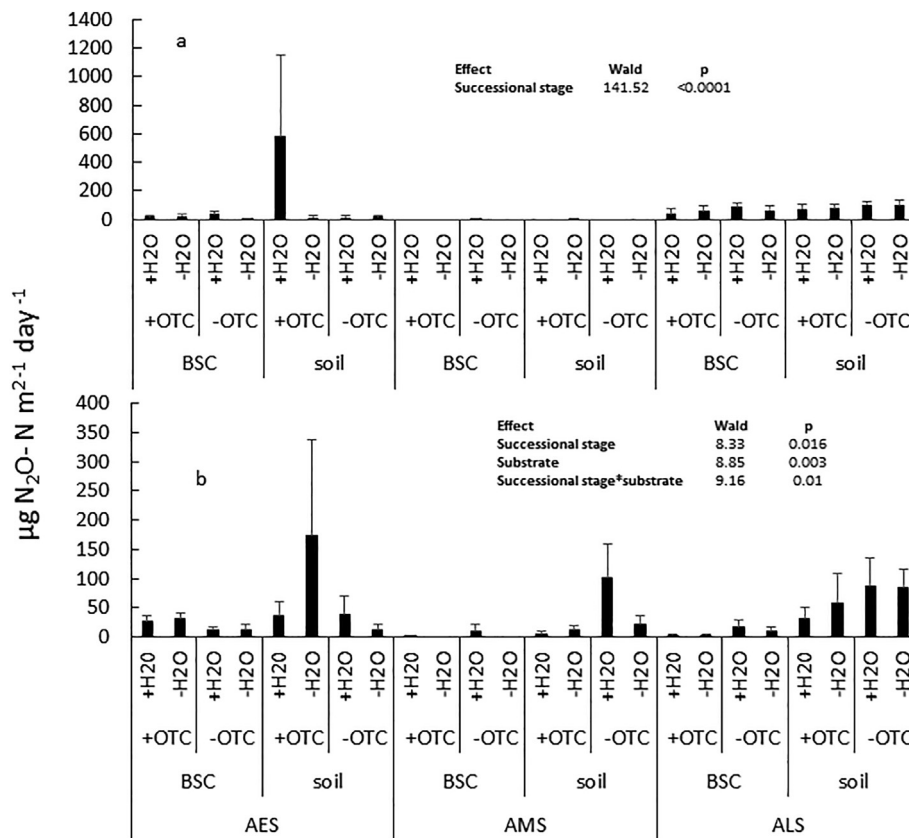

Fig. 3. Mean (n = 6 ± SD) denitrification rates in the three successional stages of the Ardley chronosequence in the BSC and soil inside (+OTC) and outside (−OTC) the open-top chambers and with (+H_2O) and without (−H_2O) water addition, during (a) February 2019 and (b) February 2021. Only the significant effects of Wald statistics are shown in the box (Tables 6S and7S).

difference in denitrification rates between BSCs and soils was higher in ALS than in AMS and AES (Fig. 3b, Table 7S).

In Barton Peninsula in 2019 the only significant effect on denitrification rates was the successional stage, and higher rates were found in BMS than in BLS (Fig. 4a, Table 8S). There was no effect of substrate, passive warming or water addition (Table 7S). In 2021, no effect was detected on denitrification rates, however the trend towards higher rates in BMS was maintained (Fig. 4b, Table 9S).

3.5. Relative abundance and structure of soil bacterial communities

The most abundant phyla of soil bacteria (>10%) in both chronosequences were *Acidobacteria*, *Proteobacteria*, *Bacteroidetes* and *Planctomycetes* (data not shown). The classes of soil bacteria that increased in abundance (AES < AMS < ALS) over the Ardley chronosequence were *Acidobacteriia*, *Solibacteres* (*Acidobacteria*) and PAC001852_c (*Acidobacteria*). Of the most abundant classes, decreases (AES > AMS > ALS) were observed in α-*Proteobacteria*, *Sphingobacteriia* (*Bacteroidetes*) and *Spartobacteria* (*Verrucomicrobia*) (Fig. 5a). In the Barton chronosequence, the classes that increased (BES < BMS < BLS) were *Planctomycetia* and *Solibacteres*, while decreases (BES > BMS > BLS) were observed in *Sphingobacteriia*, γ-Proteobacteria, β-Proteobacteria and Actinobacteria_c. The class *Acidobacteriia* were most abundant in BMS (Fig. 5b). Archaeal sequences consisted of only 0.03% of the total 16S reads, with mostly belonging to ammonia oxidizing genus *Nitrososphaera* (Thaumarchaeota).

In the Ardley chronosequence, ALS presented the lowest α-diversity of soil bacterial communities ($F_{2,11} = 8.94$; $p < 0.005$, Fig. 6a). In the Barton chronosequence, BLS presented the highest α-diversity of soil bacterial communities ($F_{2,12} = 9.56$; $p < 0.03$, Fig. 6b). The β-diversity indicates a gradual shift in the bacterial community structure along the chronosequence in Ardley Island (Fig. 7a), while in Barton Peninsula the three different successional sites are more dissimilar (Fig. 7b).

4. Discussion

4.1. Patterns of diazotrophic activity

During the 2 years of the study, both the successional stage and the substrate were found to present significant effects on diazotrophic activity in both chronosequences. The effects were significantly lower in soil than in BSCs. The higher rates found in BSCs are explained by the fact that our measurements of acetylene reduction activity included free-living microorganisms, endophytes in lichens and soil crusts, and even epiphyll in mosses. Abundant N-fixing α-Proteobacteria have been found in the *Usnea*-moss association and in *Cladonia* and *Ochrolechia* lichens of maritime Antarctica (Lee et al., 2014; Park et al., 2016). In soil, on the other hand, only free-living symbiotic nitrogen fixation is measured.

In the Ardley chronosequence, the earliest stage of succession (AES) presented the lowest rates of diazotrophic activity, however in the Barton chronosequence the earliest succession stage (BES) presented the highest rate. This contrasting pattern may arise from differences in the state factors of the chronosequences, for example soil origin (raised beaches in Ardley vs. glacial melting in Barton (Fretwell et al., 2010; Seong et al., 2009)), climate (harsher climate in Barton than in Ardley, as suggested by Table 3), and the chemical properties of soils (i.e. less acidic in the Barton chronosequence, as suggested by Table 2). Under such scenarios, contrasting patterns of ecosystem development are expected (Delgado-Baquerizo et al., 2019; Delgado- Baquerizo et al., 2020). The higher rates of diazotrophic activity in BES are associated with the highest soil pH and content of base cations, and lower contents of total C and water in soils than in BMS and BLS. In BES the moss *Bryum* sp. dominates the BSCs, and bacterial association with diazotrophs probably contributes to the significant acetylene reduction activity. Similarly, plant cover in the primary succession of Surtsey Island of Iceland is dominated by the moss *Bryum*, which presents epiphyll association with N-fixing *Cyanobacteria* of the species *Nostoc*
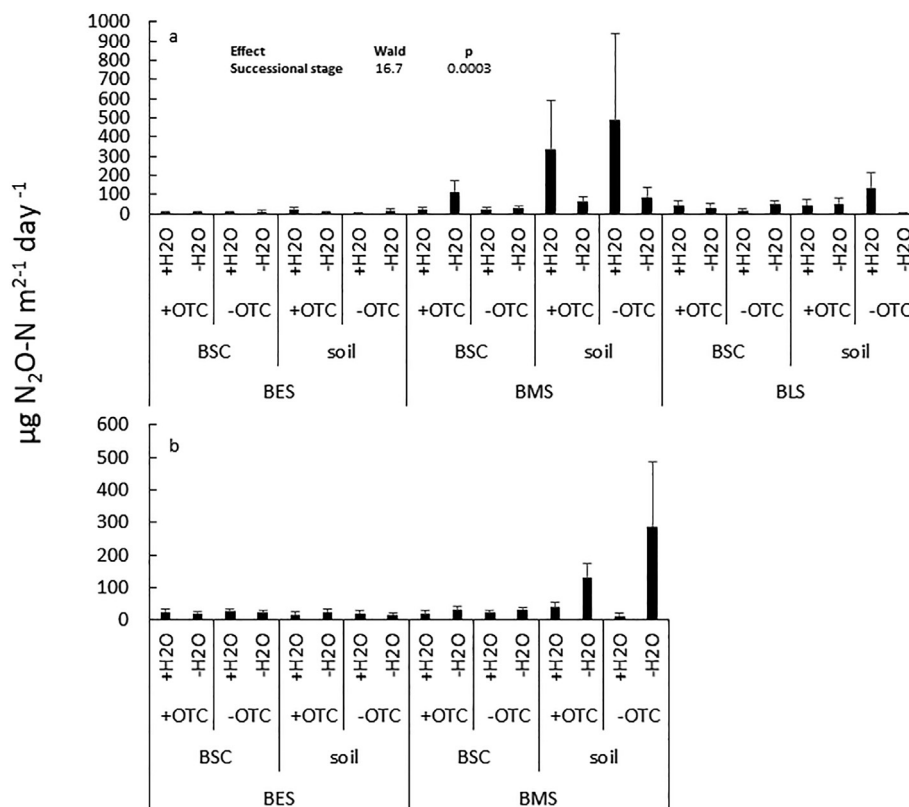

Fig. 4. Mean (n = 6 ± SD) denitrification rates in the three successional stages of the Barton chronosequence in the BSC and soil inside (+OTC) and outside (−OTC) the open-top chambers and with (+H₂O) and without (−H₂O) water addition, during (a) February 2019 and (b) February 2021. In 2021, only BES and BMS were measured. Only the significant effects of Wald statistics are shown in the box (Tables 8S and 9S).
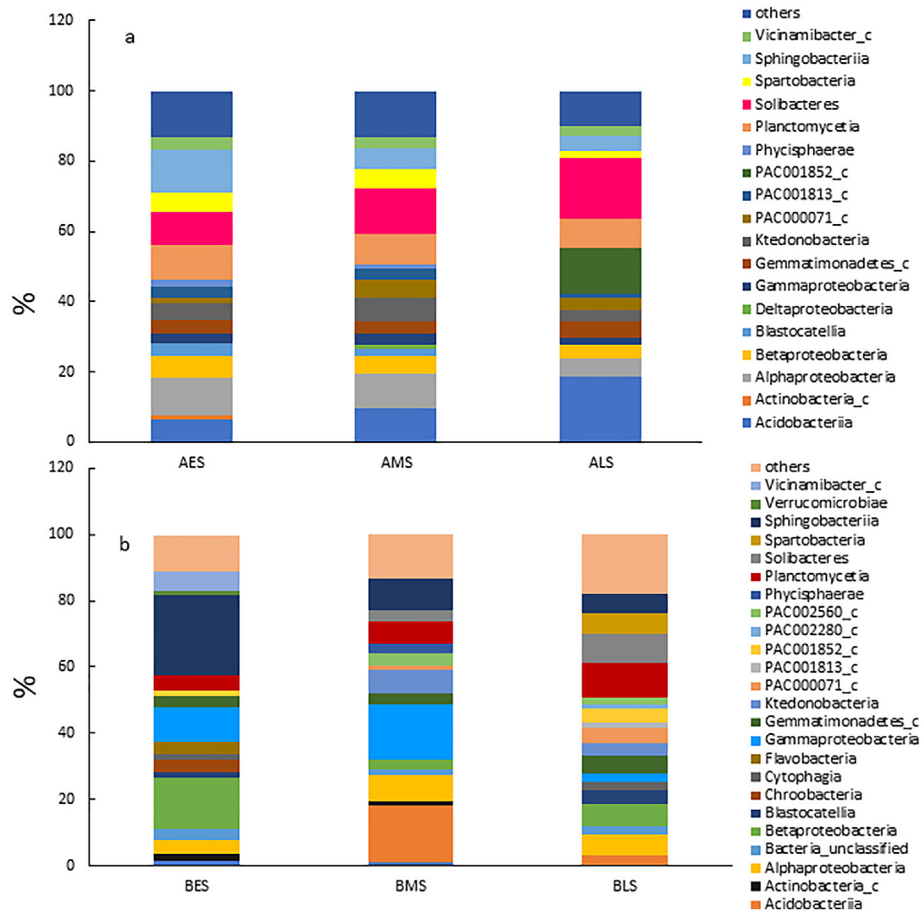
Fig. 5. Relative abundance (%) of classes of soil bacteria in three successional stages of the Ardley (a) and Barton chronosequence (b).

calcicola (Henriksson et al., 1987). Other cyanobacteria present in soils, such as *Microcoleus*, most probably form such associations with the *Bryum* species in our study (see below). In the Ardley chronosequence, a higher rate of diazotrophic activity was found in ALS, associated with a higher total P content – a limiting factor on nitrogen fixation (Reed et al., 2011).

4.2. The effect of passive warming and humidity on diazotrophic activity

In the Ardley chronosequence, positive effects of passive warming and moisture on diazotrophic activity were recorded in the first and the second years respectively. We interpret this result as follows: in the Ardley
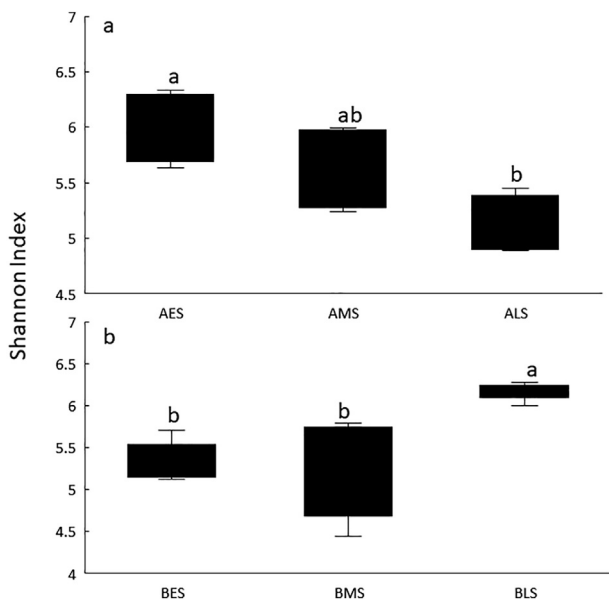

Fig. 6. Mean α-diversity ($n = 5$, Shannon-Index) of soil bacterial communities in the three successional stages of the Ardley (a) and Barton (b) chronosequences.
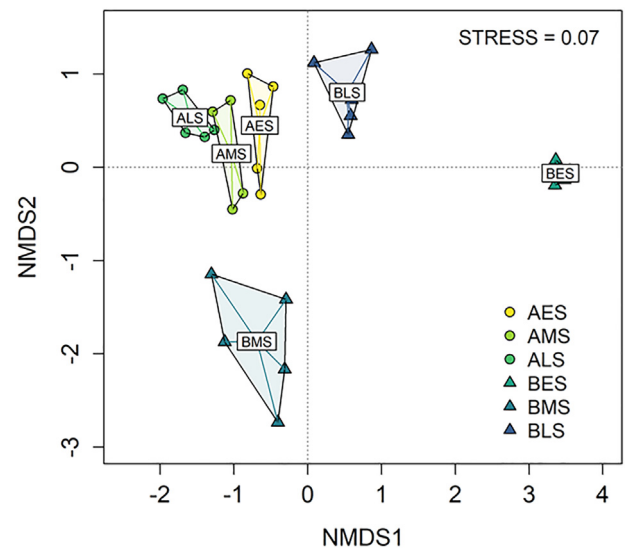

Fig. 7. β-Diversity of soil bacterial communities based on Bray–Curtis dissimilarities among all pairs of samples visualized using non-metric multidimensional scaling (NMDS). Convex hulls were added around groups of samples with connecting each sample to a group centroid.

chronosequence, during the 3-day course of the field experiments, the surface air temperature showed a contrasting difference averaging 1 °C between the treatments inside and outside the OTCs (Fig. 2aS); temperatures below zero were recorded only outside the OTCs. Moreover, the field water content of the BSCs was 60% average (Fig. 2cS). This environmental setting during the first year of the study determined that warming would become more limiting because the moisture condition was sufficient for diazotrophic activity. In the second year of the study in this chronosequence, however, the temperature difference between inside and outside the OTCs was minimal, only 0.3 °C on average, and no temperatures below zero were recorded (Fig. 2bS); in contrast the field water content of the BSCs was less than 35% on average (Fig. 2bS). Thus in the second year of the study, humidity would be a stronger limiting factor on diazotrophic activity than temperature.

In the Barton chronosequence, on the other hand, our results indicate that passive warming and moisture may not be limiting factors on diazotrophic activity, as no response was found in the first year. In the second year of the study, passive warming may even have had the effect of increasing diazotrophic activity in BMS, but without water addition, as indicated by the significant interactive effect of successional stage*passive warming*water. This was the experiment site with the highest water content, available P, and total N and C; thus, the addition of water might have produced a dilution of nutrients for diazotrophs. This line of evidence suggests that the addition of nutrients such as C, P and microelements, cofactors of the nitrogenase enzyme, may be more effective in increasing diazotrophic activity in this chronosequence than passive warming or water addition. In fact, this chronosequence presented significantly lower contents of base cations, available P and total N and C than the soils of the Ardley chronosequence. All these elements have been reported as limiting factors on diazotrophic activity (Reed et al., 2011; Pérez et al., 2017a).

4.3. Denitrification patterns

Contrary to diazotrophic activity, the highest rates of denitrification were found in the soil. This result is expected, as most denitrifiers are free-living and depend on the widely available nitrate in the soil of the ecosystem. In the Ardley chronosequence, AMS presented the lowest denitrification rate in the first year of the study; in the second year it was still lower than ALS. As the chemical parameters of the soils are similar in the three successional stages, finding a plausible explanation of this pattern remains an open question.

In the Barton chronosequence, during both years of the study BMS presented a trend towards higher denitrification rates, associated with higher contents of water, available N and P, and total C and N. In maritime Antarctica, higher denitrification rates have been found associated with higher contents of available N and C in soils (Pérez et al., 2017b; Dai et al., 2020). The fact that there was no effect of passive warming and water addition on denitrification rates suggests that these factors are not limiting on denitrification in either chronosequence.

4.4. Patterns of microbial community abundance and structure

The most abundant phyla found in the present study, such as *Acidobacteria* and *Proteobacteria*, are usually found in high abundances in maritime Antarctica (Ganzert et al., 2011, Wang et al., 2015, Garrido-Benavent et al., 2020, Krauze et al., 2021), with the phylum *Planctomycetes* also presenting important numbers. This phylum is commonly described to be abundant in *Sphagnum*-moorland and lichen-dominated tundra, and is recognized for its high hydrolytic and glycolytic capabilities (Dedysh and Ivanova, 2019). Of these, the most abundant soil bacteria, *Acidobacteria* showed a trend to increase over the succession stages from early through mid to late in the Ardley chronosequence. Similar trends have been found in several chronosequences of glacier forefields in Artic, Sub-Antarctic and glacial mountains around the globe (Zumsteg et al., 2012; Rime et al., 2015; Kwon et al., 2015; Kim et al., 2017; Jiang et al., 2018; Castle et al., 2016; Fernandez-Martinez et al., 2017), and even in the Antarctic (Krauze

et al., 2021; Garrido-Benavent et al., 2020). This trend is generally accompanied by decreasing soil pH with soil age (Zumsteg et al., 2012, Mateos-Rivera et al., 2016, Kim et al., 2017). In fact, in the Barton chronosequence the highest abundance of *Acidobacteria* is found in BMS, where the soil presented the lowest pH value. *Acidobacteria* have been defined as an oligotrophic group of bacteria with high capabilities in the decomposition of a wide variety of polysaccharides and in the production of exopolysaccharides, thereby contributing to soil stabilization (Kielak et al., 2016). *Planctomycetes* are likewise found in higher abundance in BLS, also characterized by their ability to degrade carbon from organic matter complexes such as lichenan and chitin (Dedysh and Ivanova, 2019). Therefore both *Acidobacteria* and *Planctomycetes* are important phyla contributing to organic matter decomposition and incorporation into the soil in older successional stages.

Of the most frequent classes, those that decreased in relative abundance were the α-and β-*Proteobacteria* in the Ardley and Barton chronosequences, respectively. Similar patterns were found in glacier forelands of the Damma Glacier (Zumsteg et al., 2012) and in Antarctica (Garrido-Benavent et al., 2020). The widespread presence of N-fixers in the phylum *Proteobacteria* has been documented (Nelson et al., 2016) but the decrease observed in one of the most common representative N-fixers, *Rhizobiales*, bore no relation to the increase in diazotrophic activity observed in the Ardley chronosequence (Fig. 3aS). The decrease in β-*Proteobacteria* (*Burkholderiales*) and *Actinobacteria* (*Frankiales*) in the Barton chronosequence followed the decrease in diazotrophic activity found in the present study (Fig. 3bS). A decrease in the relative abundance of *Actinobacteria* with soil age has also been observed in glacier forelands around the world (Rime et al., 2015, Kwon et al., 2015, Fernandez-Martinez et al., 2017) and in Antarctica (Krauze et al., 2021). *Cyanobacteria* is one of the diazotroph phyla that have been commonly described to be important components of bacterial assemblages in glacier forefields (Zeng et al., 2016, Fernandez-Martinez et al., 2017, Rippin et al., 2018, Pessi et al., 2019). In the Ardley chronosequence, we found that the relative abundance of *Cyanobacteria* presented a trend to increase towards the latest successional stage (Fig. 3aS), a trend also found in a glacier forefield on Livingstone Island in Antarctica (Garrido-Benavent et al., 2020) and Svalbard in the Arctic (Pessi et al., 2019). However, in our study there was no representative of any genus of either heterocyst or non-heterocyst N-fixing *Cyanobacteria*, except for *Microcoleus* (0.05%) present in AES (data not shown); this is because 85% of the ASVs of *Cyanobacteria* could not be assigned to any known genera, and these probably account for the high diazotrophic activity found in AMS and ALS. In the Barton chronosequence, several non-heterocyst, N-fixing *Cyanobacteria* bacteria were found exclusively in BES. *Microcoleus* (2.2%) was found in high abundance, with lesser abundance (< 0.01%) of *Chamaesiphon*, *Chroococcidiopsis*, *Leptolyngbya*, *Nostoc* (heterocyst) and *Oscillatoria* (data not shown). Most probably one of these abundant free-living soil bacteria (e.g., *Microcoleus*) finds a suitable habitat in the moist microenvironment of the *Bryum* phyllids, thus forming an epiphyllic association, contributing to the high rates of diazotrophic activity found in the BSCs of BES. Further research is needed in order to confirm this hypothesis. In both chronosequences, Ardley and Barton, the relative abundance of *Sphingobacteria* (*Bacteroidetes*) decreased over the succession stages from early through mid to late, similar to findings in the Hailuogou Glacier chronosequence (Jiang et al., 2018). *Sphingobacteria* have been found to increase in abundance as rising temperatures have increased available C in the permafrost soils of the Arctic Tundra (Ricketts et al., 2020). In our case, the higher abundance of *Sphignobacteria* in early successional soils may be associated with a higher proportion of newly available organic C (probably from the nearby glacier or ocean inputs), rather than with more recalcitrant forms of C coming from lichen plant material accumulated over thousands of years of soil development.

Denitrifiers are also important soil bacteria found in ice-free regions of maritime Antarctica (Zhu et al., 2009; Dai et al., 2020; Ramírez-Fernández et al., 2021). In our study, the most abundant denitrifier belongs to the genus *Rhodanobacter*; it represented up to 10% in BMS, where it is associated with a trend towards the highest denitrification rates (Fig. 4bS). This genus has already been reported to be abundant in ornithogenic soils

of maritime Antarctica (Ramírez-Fernández et al., 2021). Moreover, the changes in the composition of denitrifiers in the three successional stages, with the genus *Thiobacillus* predominating in BES, suggests that a diversity of groups is performing this function. Other denitrifiers present important abundances in the Ardley chronosequence, such as *Rhizobacter* in ALS (Fig. 4aS). In AMS, *Rhodanobacter* has the lowest abundance associated with lower denitrification rates (Fig. 4aS).

Studies performed in glacier forefields report either increases, decreases or neutral effects on bacterial α-diversity. In the present study we found higher bacterial diversity in the early successional site of the Ardley chronosequence, whereas in the Barton chronosequence the highest bacterial diversity was found in the late successional site. Other studies performed in glacier forefields of maritime Antarctica report that bacterial α-diversity increases with distance from the glacier terminus (Garrido-Benavent et al., 2020, Krauze et al., 2021), associated with a decrease in pH values and an increase in soil organic matter in well vegetated sites. However, Bajerski and Wagner (2013), in the Larsemann Hills of East Antarctica, found the lowest α-diversity in the site most distant from the glacier terminus, linked to lower pH and C content in soils than at the site closer to the glacier. These results suggest that the factors affecting microbial diversity may vary from one glacier forefield to another, depending on other unconsidered factors such as the source of microbial inputs from the ocean and glacier runoff. It may be noted that the highest α-diversity in the Barton chronosequence was found precisely in the oldest soil in BLS, with low contents of nutrients and – even more noteworthy – with the lowest mean surface annual temperature. Here in this harsh environment, the high soil α-diversity may be explained by the increase in protective plant cover, and probably also by the higher variety of organic compounds with soil age (Delgado-Baquerizo et al., 2019).

The β-diversity in the Ardley chronosequence indicates a gradual change in the soil microbial assemblage from the younger to the older successional stages. In the Barton chronosequence in contrast, there is marked dissimilarity between the three successional stages; this suggests that other contrasting factors between the successional stages apart from soil age – such as soil type and microclimate – combine to create greater differences in the soil microbial assemblages in the different successional stages.

5. Conclusions

In the present study we found contrasting patterns of microbial activity and soil microbial community structure in the two chronosequences studied. These may be a consequence of their different environmental settings, mainly differences in soil origin (raised beaches vs. glacial melting) and climatic conditions (mild vs. harsher climate). Moreover, the different responses to passive warming and water addition may express the different degrees of limitation on microbial activity caused by warming and moisture. Finally, interannual climatic variability, which determined the moisture of the substrate and the temperature conditions during the experimental setting, was also reflected in a differential response of microbial activity to either warming or water addition. However, in the long term, the predicted increases in air temperature and precipitation in maritime Antarctica will produce strong positive effects on diazotrophic activity where sufficient nutrients are available. So far it is not possible to predict the trends in denitrification rates, as these appear to depend on nutrient availability.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152234.

References

Alfaro, F.D., Salazar-Burrows, A., Bañales-Seguel, C., García, J.L., Manzano, M., Ruz, K., Gaxiola, A., 2020. Soil microbial abundance and activity across forefield glacier chronosequence in the Northern Patagonian Ice Field, Chile. Arct. Antarct. Alp. Res. 52, 553–562.

Bajerski, F., Wagner, D., 2013. Bacterial succession in Antarctic soils of two glacier forefields on Larsemann Hills, East Antarctica. FEMS Microbiol. Ecol. 85, 128–142.

Boy, J., Godoy, R., Shibistova, O., Boy, D., McCulloch, R., de la Fuente, R.A., Aguirre, M., Mikutta, R., Guggenberger, G., 2016. Successional patterns along soil development gradients formed by glacier retreat in the maritime Antarctic, King George Island. Rev. Chil. Hist. Nat. 89, 6.

Bozkurt, D., Bromwich, D.H., Carrasco, J., Rondanelli, R., 2021. Temperature and precipitation projections for the Antarctic peninsula over the next two decades: contrasting global and regional climate model simulations. Clim. Dyn. 56, 3853–3874.

Bradley, J.A., Singarayer, J.S., Alexandre, M., Anesio, A.M., 2014. Microbial community dynamics in the forefield of glaciers. Proc. R. Soc. B 281, 20140882.

Brunner, E., Konietschke, F., Pauly, M., Puri, M.L., 2017. Rank-based procedures in factorial designs: hypotheses about non-parametric treatment effects. J. R. Stat. Soc. Ser. B Stat Methodol. 79 (5), 1463–1485.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583.

Castle, S.C., Nemergut, D.R., Grandy, A.S., Leff, J.W., Graham, Hood, E., Schmidt, S.K., Wickings, K., Cleveland, C.C., 2016. Biogeochemical drivers of microbial community convergence across actively retreating glaciers. Soil Biol. Biochem. 101, 74–84.

Cofaigh, C.O., Davies, B.J., Livingstone, S.J., Smith, J.A., Johnson, J.S., Hocking, E.P., Hodgson, D.A., Anderson, J.B., Bentley, M.J., Canals, M., Domack, E., Dowdeswell, J.A., Evans, J., Glasser, N.F., Hillenbrand, C.D., Larter, R.D., Roberts, S.J., Simms, A.R., 2014. Reconstruction of ice-sheet changes in the Antarctic peninsula since the last glacial maximum. Quat. Sci. Rev. 100, 87–110.

Dai, H.-T., Ren-Bin Zhu, R.-B., Sun, B.-W., Che, C.-S., Hou, L.-J., 2020. Effects of sea animal activities on tundra soil denitrification and nirS- and nirK-encoding denitrifier community in maritime Antarctica. Front. Microbiol. 11, 573302.

Dedysh, S.N., Ivanova, A.A., 2019. Planctomycetes in boreal and subarctic wetlands: diversity patterns and potential ecological functions. FEMS Microbiol. Ecol. 95.

Delgado- Baquerizo, M., Reich, P.B., Bardgett, R.D., Eldridge, D.J., Lambers, H., Wardle, D.A., Reed, S.C., Plaza, C., Png, G.K., Neuhauser, S., Berhe, A.A., Hart, S.C., Hu, H.W., He, J.Z., Bastida, F., Abades, S., Alfaro, F.D., Cutler, N.A., Gallardo, A., García-Velázquez, L., Hayes, P.E., Hseu, Z.Y., Pérez, C.A., Santos, F., Siebe, C., Trivedi, P., Sullivan, B.W., Weber-Grullon, L., Williams, M.A., Fierer, N., 2020. The influence of soil age on ecosystem structure and function across biomes. Nat. Commun. 11, 4721.

Delgado-Baquerizo, M., Bardgett, R.D., Vitousek, P., Maestre, F.T., Williams, M.A., Eldridge, D.J., Lambers, H., Neuhauser, S., Gallardo, A., García-Velázquez, L., Sala, O.E., Abades, S.R., Alfaro, F.D., Berhe, A.A., Bowker, M.A., Currier, C.M., Cutler, N.A., Hart, S.C., Hayes, P.E., Hseu, Z.Y., Kirchmair, M., Peña-Ramírez, V.M., Pérez, C.A., Reed, S.C., Santos, F., Siebe, C., Sullivan, B.W., Weber-Grullon, L., Fierer, N., 2019. Changes in belowground biodiversity during ecosystem development. PNAS 116, 6891–6896.

Fernandez-Martinez, M.A., Perez-Ortega, Pointing, S.B., Green, T.G.A., Pintado, A., Rozzi, R., Sancho, L.G., De los Ríos, A., 2017. Microbial succession dynamics along glacier forefield chronosequences in Tierra del Fuego (Chile). Polar Biol. 40, 1939–1957.

Fretwell, P.T., Hodgson, D.A., Watcham, E.P., Bentley, M.J., Roberts, S.J., 2010. Holocene isostatic uplift of the South Shetland Islands, Antarctic Peninsula, modelled from raised beaches. Quat. Sci. Rev. 29, 1880–1893.

Ganzert, L., Lipski, A., Hubberten, H.-W., Wagner, D., 2011. The impact of different soil parameters on the community structure of dominant bacteria from nine different soils located on Livingston Island, South Shetland Archipelago, Antarctica. FEMS Microbiol. Ecol. 76, 476–491.

Garrido-Benavent, I., Pérez-Ortega, S., Durán, J., Ascaso, C., Pointing, S.B., Rodríguez-Cielos, R., Navarro, F., de los Ríos, A., 2020. Differential colonization and succession of microbial communities in rock and soil substrates on a maritime Antarctic glacier forefield. Front. Microbiol. 11, 126.

Göransson, H., Venterink, H.O., Bååth, E., 2011. Soil bacterial growth and nutrient limitation along a chronosequence from a glacier forefield. Soil Biol. Biochem. 43, 1333–1340.

Groffman, P.M., Holland, E.A., Myrold, D.D., Robertson, G.P., Zou, X., 1999. Denitrification. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 241–257.

Gyeong, H., Hyun, C.U., Kim, S.C., Tripathi, B.M., Yun, J., Kim, J., Kim, S., Kim, M., 2021. Contrasting early successional dynamics of bacterial and fungal communities in recently deglaciated soils of the maritime Antarctic. Mol. Ecol. 4231–4244.

Henriksson, E., Henriksson, L.E., Norrman, J.O., Nyman, P.O., 1987. Biological dinitrogen fixation (acetylene reduction) exhibited by blue-green algae (Cyanobacteria) in association with mosses gathered on Surtsey Iceland. Arct. Alp. Res. 19, 432–436.

Jiang, Y., Lei, Y., Yang, Y., Korpelainen, H., Niinemets, Ü., Li, Ch., 2018. Divergent assemblage patterns and driving forces for bacterial and fungal communities along a glacier forefield chronosequence. Soil Biol. Biochem. 118, 207–216.

Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., van Veen, J.A., Kuramae, E.E., 2016. The ecology of acidobacteria: moving beyond genes and genomes. Front. Microbiol. 7, 744.

Kim, M., Jung, J.Y., Laffly, D., Kwon, H.Y., Lee, Y.K., 2017. Shifts in bacterial community structure during succession in a glacier foreland of the high Arctic. FEMS Microbiol. Ecol. 93, fiw213.

Krauze, P., Wagner, D., Spinola, D.N., Kühn, P., 2021. Influence of prokariotic microorganisms on initial soil formation along a glacier forefield on King George Island, maritime Antarctica. Sci. Rep. 11, 13135.

Kuo, S., 1996. Phosphorus. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods. SSSA and ASA, Madison, WI, pp. 869–919.

Kwon, H.Y., Jung, J.Y., Kim, O.-S., Laffly, D., Lim, H.S., Lee, Y.K., 2015. Soil development and bacterial community shifts along the chronosequence of the Midtre Lovénbreen glacier foreland in Svalbard. J. Ecol. Environ. 38, 461–476.

Lee, J., Jin, Y.K., Hong, J.K., Yoo, H.J., Shon, H., 2008. Simulation of a tidewater glacier evolution in Marian Cove, king George island, Antarctica. Geosci. J. 12, 33–39.

Lee, Y.M., Kim, E.H., Lee, H.K., Hong, S.G., 2014. Biodiversity and physiological characteristics of Antarctic and Arctic lichens-associated bacteria. World J. Microbiol. Biotechnol. 30, 2711–2721.

Lopes, D., Schaefer, C.E.G.R., Souza, J.J.L.L., Oliveira, F.S., Simas, F.N.B., Daher, M., Gjorup, D.F., 2019. Concretionary horizons, unusual pedogenetic processes and features of sulfate affected soils from Antarctica. Geoderma 347, 13–24.

Mateos-Rivera, A., Yde, J.C., Wilson, B., Finster, K.W., Lise Øvreas, L., Reigstad, L.J., 2016. The effect of temperature change on the microbial diversity and community structure along the chronosequence of the sub-arctic glacier forefield of Styggedalsbreen (Norway). FEMS Microbiol. Ecol. 92.

Michel, R.F.M., Schaefer, C.E.G.R., López-Martínez, J., Simas, F.N.B., Haus, N.W., Serrano, E., Bockheim, J.G., 2014. Soils and landforms from Fildes Peninsula and Ardley Island, Maritime Antarctica. Geomorphology 225, 76–86.

Mori, A.S., Osono, T., Cornelissen, J.H.C., Craine, J., Uchida, M., 2017. Biodiversity–e ecosystem function relationships change through primary succession. Oikos 126, 1637–1649.

Myrold, D.D., Ruess, R.R., Klug, M.J., 1999. Dinitrogen fixation. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 241–257.

Nelson, M.B., Martiny, A.C., Martiny, J.B.H., 2016. Global biogeography of microbial nitrogen-cycle traits in soil. PNAS 113, 8033–8044.

Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18 (5), 1403–1414.

Park, C.H., Kim, K.M., Kim, O.-S., Jeong, G., Hong, S.G., 2016. Bacterial communities in Antarctic lichens. Antarct. Sci. 28, 455–461.

Pérez, C.A, Hedin, L.O., Armesto, J.J., 1998. Nitrogen mineralization in two unpolluted old-growth forests of contrasting biodivesity and dynamics. Ecosystems 1, 361–373.

Pérez, C.A., Aravena, J.C., Mc Culloch, R., Ivanovich, C., 2017b. Effects of penguin guano and moisture on biological fixation of nitrogen in maritime Antarctic soils. Polar Biol. 40, 437–448.

Pérez, C.A., Thomas, F.M., Silva, W.A., Aguilera, R., Armesto, J.J., 2017a. Biological nitrogen fixation in a post-volcanic chronosequence from south-central Chile. Biogeochemistry 132, 23–36.

Pessi, I.S., Osorio-Forero, C., Gálvez, E.J., Simões, F.L., Simões, J.C., Junca, H., Macedo, A.J., 2015. Distinct composition signatures of archaeal and bacterial phylotypes in the Wanda glacier forefield, Antarctic Peninsula. FEMS Microbiol. Ecol. 91.

Pessi, I.S., Pushkareva, E., Lara, Y., Borderie, F., Wilmotte, A., Elster, J., 2019. Marked succession of cyanobacterial communities following glacier retreat in the high Arctic. Microb. Ecol. 77, 136–147.

Peter, H.-U., Buesser, C., Mustafa, O., Pfeiffer, S., 2008. Risk assessment for the fildes peninsula and Ardley Island and the development of management plans for designation as Antarctic specially protected or managed areas. Fed. Environ. Agency Texte 20 (8), 508.

Pickett, S.T.A., 1989. Space-for-time substitution as an alternative to long-term studies. In: Likens, G.E. (Ed.), Long-term Studies in Ecology. Springer, New York, NY.

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press, New York.

Ramírez-Fernández, L., Orellana, L.H., Johnston, E.R., Konstantinidis, K.T., Orlando, J., 2021. Diversity of microbial communities and genes involved in nitrous oxide emissions in Antarctic soils impacted by marine animals as revealed by metagenomics and 100 metagenome-assembled genomes. Sci. Total Environ. 788, 147693.

Reed, S.C., Cleveland, C.C., Townsend, A.R., 2011. Functional ecology of free-living nitrogen fixation: a contemporary perspective. Annu. Rev. Ecol. Syst. 42, 489–512.

Ricketts, M.P., Matamala, R., Jastrow, J.D., Antonopoulos, D.A., Koval, J., Ping, C.-L., Liang, C., Gonzalez-Meler, M.A., 2020. The effects of warming and soil chemistry on bacterial community structure in Arctic tundra soils. Soil Biol. Biochem. 148, 107882.

Rime, T., Hartmann, M., Brunner, I., Widmer, F., Zeyer, J., Frey, B., 2015. Vertical distribution of the soil microbiota along a successional gradient in a glacier forefield. Mol. Ecol. 24, 1091–1108.

Rippin, M., Borchhardt, N., Williams, L., Colesie, C., Jung, P., Büdel, B., Karsten, U., Becker, B., 2018. Genus richness of microalgae and cyanobacteria in biological soil crusts from Svalbard and Livingston Island: morphological versus molecular approaches. Polar Biol. 41, 909–923.

Robertson, G.P., Sollins, P., Ellis, B., Lajtha, K., 1999. Exchangeable ions, pH, and cation exchange capacity. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 106–114.

Rückamp, M., Braun, M., Suckro, S., Blindow, N., 2011. Observed glacial changes on the King George Island ice cap, Antarctica, in the last decade. Glob. Planet. Change 79, 99–109.

Sáez, P.L., Cavieres, L.A., Galmés, J., Gil-Pelegrín, E., Peguero-Pina, J.J., Sancho-Knapik, D., Vivas, M., Sanhueza, C., Ramírez, C.F., Rivera, B.K., Corcuera, L.J., Bravo, L.A., 2018. In situ warming in the Antarctic: effects on growth and photosynthesis in Antarctic vascular plants. New Phytol. 218, 1406–1418.

Schurig, C., Smittenberg, R.H., Berger, Kraft, J., Woche, F.K., Goebel, M.O., Heipieper, H.J., Miltner, A., Kaestner, M., 2013. Microbial cell-envelope fragments and the formation of soil organic matter: a case study from a glacier forefield. Biogeochemistry 113, 595–612.

Seong, Y.B., Owen, L.A., Lim, H.S., Yoon, H.I., Kim, Y., Lee, Y.I., Caffee, M.W., 2009. Rate of late quaternary ice-cap thinning on King George Island, South Shetland Islands, West Antarctica defined by cosmogenic 36Cl surface exposure dating. Boreas 38, 207–213.

Sollins, P., Glassman, C., Paul, E.A., Swanston, C., Lajtha, K., Heil, J.W., Elliot, E.T., 1999. Soil carbon and nitrogen: pools and fractions. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 106–114.

Strauss, S.L., Ruhland, C.T., Day, T., 2009. Trends in soil characteristics along a recently deglaciated foreland on Anvers Island, Antarctic Peninsula. Polar Biol. 32, 1779–1788.

Wang, N.F., Zhang, T., Zhang, F., Wang, E.T., He, J.F., Ding, H., Zhang, B.T., Liu, J., Ran, X.B., Zang, J.Y., 2015. Diversity and structure of soil bacterial communities in the fildes region (maritime Antarctica) as revealed by 454 pyrosequencing. Front. Microbiol. 6, 1188.

Watcham, E.P., Bentley, M.J., Hodgson, D.A., Roberts, S.J., Fretwell, P.T., Lloyd, J.M., Larter, R.D., Whitehouse, P.L., Leng, M.J., Monien, P., Moreton, S.G., 2011. A new Holocene relative sea level curve for the South Shetland Islands, Antarctica. Quat. Sci. Rev. 30, 3152–3170.

Yan, W., Ma, H., Shi, G., Li, Y., Sun, B., Xiao, X., Zhang, Y., 2017. Independent shifts of abundant and rare bacterial populations across East Antarctica glacial foreland. Front. Microbiol. 8, 1534.

Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67 (5), 1613.

Zeng, J., Lou, K., Zhang, C.-J., Wang, J.-T., Hu, H.-W., Shen, J.-P., Zhang, L.-M., Han, L.-L., Zhang, T., Lin, Q., Chalk, P.M., He, J.-Z., 2016. Primary succession of nitrogen cycling microbial communities along the deglaciated forelands of Tianshan Mountain, China. Front. Microbiol. 7, 1353.

Zhu, R., Liu, Y., Ma, E., Sun, J., Xu, H., Sun, L., 2009. Nutrient compositions and potential greenhouse gas production in penguin guano, ornithogenic soils and seal colony soils in coastal Antarctica. Antarct. Sci. 21, 427.

Zumsteg, A., Luster, J., Göransson, H., Smittenberg, R.H., Brunner, I., Bernasconi, S.M., Josef Zeyer, J., Frey, B., 2012. Bacterial, archaeal and fungal succession in the forefield of a receding glacier. Microb. Ecol. 63, 552–564.